

## Original Report

# Prevalence of Intestinal Parasitic Infections in Patients with Acquired Immunodeficiency Syndrome in Brazil

Sérgio Cimerman, MD, MSc;\* Benjamin Cimerman, MD, MSc;† and David Salomão Lewi, MD, PhD‡

## ABSTRACT

**Objectives:** To evaluate the prevalence of intestinal parasitic infections and to investigate the possible associations of clinical status and laboratory findings with the different parasites found in stool samples.

**Methods:** Each patient was provided with one standard fecal collection vial containing 10% formalin for detecting ova, larvae, and cysts. To detect *Cryptosporidium parvum* and *Isospora belli*, the acid-fast Kinyoun stain and fluorescent auramine-rhodamine stain were used.

**Results:** A total of 200 patients with acquired immunodeficiency syndrome participated in this study; 40% were infected with at least one pathogenic species. The total prevalence of parasites was 16% for *Giardia lamblia*, 13% for *Entamoeba coli*, 7% for *Cryptosporidium parvum*, 3.5% for *Endolimax nana*, 2.5% for *Ascaris lumbricoides*, 2.5% for *Strongyloides stercoralis*, 2% for *Isospora belli*, and 0.5% for *Blastocystis hominis*. Results showed that diarrhea was significantly associated with cryptosporidiosis, giardiasis, and isosporiasis. However, no association was observed between the CD4+ cell counts and the manifestation of any particular parasite.

**Conclusions:** The data support the value of standard fecal examinations in human immunodeficiency virus-infected patients, even in the absence of diarrhea, since these examinations easily can be performed, with low costs, and frequently disclose treatable conditions.

**Key Words:** Brazil, diarrhea, HIV, parasites

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Infections caused by intestinal parasites are widespread.<sup>1</sup> Current assessments suggest that at least 25% of the world's population is chronically infected by enteric

parasites, and most of these patients live in the developing countries.<sup>2</sup>

Since the first described cases of acquired immunodeficiency syndrome (AIDS), a high prevalence of gastrointestinal infections has been reported, including diarrhea associated with parasitosis. Thus, infections in the gastrointestinal tract play a fundamental role in the morbidity and mortality of AIDS. The incidence of these infections is 50% in the developed countries, whereas it reaches up to 95% in developing countries, such as Haiti and some countries in Africa.<sup>3,4</sup> Diarrhea caused by parasites is prominent in patients with AIDS, and the occurrence of opportunistic infections caused by enteric parasites varies according to the geographic area and endemicity of each region.

The progressive decline of the mucosal immunologic defense mechanisms predisposes patients to precocious, intermediate, or late gastrointestinal manifestations.<sup>5</sup> In the late stages, the alterations of the nonspecific defense mechanisms, production of IgA antibodies, and local cellular immune responses also progress, thus increasing the susceptibility to various intestinal opportunistic agents, such as *Cryptosporidium parvum*, *Isospora belli*, and *Microsporidia* species.<sup>6</sup> After the emergence of AIDS, these parasites, known until then solely in veterinary medicine, were no longer considered commensal organisms, and they currently are recognized as common opportunist pathogens affecting human immunodeficiency virus (HIV)-infected patients, constituting a major secondary aggravating factor of the disease. These enteric infections frequently cause severe diarrhea, which often is responsible for the grievousness of the disease, and may sometimes lead to death.

The purpose of this study was to evaluate the prevalence of intestinal parasitic infections in adult patients with AIDS and to investigate the clinical, laboratory, and epidemiologic characteristics associated with the different parasitic infections.

## METHODS

### Patients

From September 1996 to April 1997, 200 HIV-positive patients attending either the Hospital of the Universidade Federal do Estado de São Paulo (Infectious and Parasitic

\*Center for Immunological Deficiencies Control, Universidade Federal do Estado de São Paulo; Instituto de Infectologia Emílio Ribas, †Department of Parasitology of the Universidade de Mogi das Cruzes, and ‡Infectious and Parasitary Diseases Unit and Center for Immunological Deficiencies Control, Universidade Federal do Estado de São Paulo, São Paulo, Brazil.

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Address correspondence to Dr. Sérgio Cimerman, Alameda Jauaperi, 890 apt 111, São Paulo, Brazil Cep. 04523-014. E-mail: sc@mandic.com.br.

**Table 1.** Prevalence of Enteropathogenic Infections in 200 Patients with AIDS

Enteropathogens	Number of Patients (n = 200) (%)	
Infected (total)*	80	(40.0)
<i>Giardia lamblia</i>	32	(16.0)
<i>Entamoeba coli</i>	26	(13.0)
<i>Cryptosporidium parvum</i>	14	(7.0)
<i>Endolimax nana</i>	7	(3.5)
<i>Ascaris lumbricoides</i>	5	(2.5)
<i>Strongyloides stercoralis</i>	5	(2.5)
<i>Isospora belli</i>	4	(2.0)
<i>Blastocystis hominis</i>	1	(0.5)
Negative	120	(60.0)

\* Data refer to patients with one or more identified parasites.

Diseases Unit and Center for Immunological Diseases Control) or the Instituto de Infectologia Emílio Ribas (IIER) facilities were prospectively enrolled in this study. The patients were informed previously about their participation in this clinical study and completed a consent document that was then approved by the Medical Ethics Committee of the two hospitals involved in this investigation. The patients also completed a short questionnaire that included name, age, risk behavior, ethnicity, weight, height, and so on. The CD4+ T cell counts, measured by flow cytometry, were obtained by reviewing patients' charts.<sup>7</sup>

The inclusion criteria to participate in this study were: age over 18 years; belonging to any HIV risk category; presenting CD4+ cell counts less than 200 cells/mm<sup>3</sup>, measured by flow cytometry, during more than 4 months; and the presence of anti-HIV antibodies, measured by enzyme-linked immunosorbent assay (ELISA) and usually confirmed by the Western-blot technique.

### Laboratory Methods

One stool sample was collected from each patient. Each participant was provided with one standard fecal collection vial containing 10% formalin (5 mL) and a spatula. Approximately 10 g of stool was collected for the parasitologic evaluation. All specimens were processed according to methods for detecting ova, larvae, and cysts devised by Hoffman et al, by Faust et al, and by Rugai et al, as described elsewhere.<sup>8</sup> To detect *C. parvum* and *I. belli* from the other intestinal protozoa, the acid-fast Kinyoun stain and the fluorescent auramine-rhodamine stain were used.<sup>9,10</sup>

The categoric data were compared using contingency table analyses with either the chi-square statistic or the Fisher's exact test. The Mann-Whitney test was applied to the quantitative data comparisons. Statistical significance was defined as  $P < 0.05$ .

### RESULTS

The age of the patients ranged from 20 to 67 years, with a median of 34 years. One hundred and thirty-nine (69.5%) patients were male and 61 were (30.5%) female. Almost all patients (99%) were living in São Paulo, Brazil. The major risk factors for acquiring HIV were related to sexual activity: promiscuous heterosexuality (n = 103; 51.5%), homosexuality (n = 40; 20%), and bisexuality (n = 13; 6.5%). Intravenous drug use was the risk factor for 37 patients (18.5%), blood transfusions for 9 (4.5%), hemophilia for 5 (2.5%), and unknown risk factor for 6 (3%).

Enteropathogens were found in the stool samples of 80 patients (40%) and some of them (5.5%) were infected with two or more intestinal parasites. *Giardia lamblia* was the most conspicuous parasite in patients with multiple infections as well as in monoparasitized individuals. Besides *G. lamblia*, the most frequently found pathogens were *Entamoeba coli* and *C. parvum* (Table 1). Other pathogens found in these Brazilian HIV-infected patients included *Endolimax nana*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Isospora belli*, and *Blastocystis hominis*.

To better visualize and differentiate the oocyst forms of *C. parvum* and *I. belli*, special staining techniques, such as Kinyoun and auramine-rhodamine stains, were applied. Oocysts from *C. parvum* stained with a brilliant red, against a blue background, and had 4- to 6- $\mu$ m rounded and uniform aspect. The oocyst forms of *I. belli* presented an oval shape averaging 8 to 11  $\mu$ m, and sometimes their spheroblasts were stained an intense red.

The mean CD4+ T cell count in this patient group was  $88.11 \pm 62.18$  cells/mm<sup>3</sup>, and the cell counts varied from 1 to 199 cells/mm<sup>3</sup>. However, no association was evidenced between the level of CD4+ T cells and the presence of enteric parasites (Table 2). The CD4+ cell counts in the patients with negative parasitologic examination was  $97.75 \pm 58.90$ , and those infected by enteropathogenic organisms had  $76.65 \pm 65.50$  CD4+ cells/mm<sup>3</sup> (Mann-Whitney test = 3888.5;  $P = 0.23$ ).

Among the 200 studied samples, 45 (22.5%) were classified as diarrheic stools and 155 (77.5%) were not;

**Table 2.** Positive and Negative Stool Examinations by Total CD4+ T Cell Count

Stool Exam	CD4+ T Cells (cells / mm <sup>3</sup> )	Median	Minimum	Maximum	Number of Patients
Negative	$97.75 \pm 58.90$	97.50	3.00	199.0	120
Positive	$76.65 \pm 65.50$	61.50	1.00	199.0	80

\* Mean  $\pm$  SD.

**Table 3.** Association between the Presence of Parasites and Fecal Aspect

Pathogens Found	Fecal Aspect		Fisher's Exact Test P
	Diarrheic n = 45 (%)	Nondiarrheic n = 155 (%)	
None	12 (26.67)	108 (67.74)	< 0.0001*
<i>Giardia lamblia</i>	12 (26.67)	20 (12.90)	0.0368*
<i>Entamoeba coli</i>	8 (17.78)	18 (11.61)	0.3147
<i>Cryptosporidium parvum</i>	11 (24.44)	3 (1.93)	< 0.001*
<i>Isospora belli</i>	3 (6.67)	1 (0.64)	0.0363*
<i>Endolimax nana</i>	2 (4.44)	5 (3.22)	0.6559
<i>Strongyloides stercoralis</i>	3 (6.67)	2 (1.59)	0.0764†
<i>Ascaris lumbricoides</i>	2 (4.44)	3 (1.93)	0.3140
<i>Blastocystis hominis</i>	0 (0.00)	1 (0.64)	1.0000

\* Statistically significant association ( $P < 0.05$ ); †indicative association ( $0.05 < P < 0.10$ ).

among the 120 negative stool exams, 108 (90%) were nondiarrheic and only 12 (10%) were diarrheic. Conversely, 47 (58.75%) patients with positive parasitologic results also presented this gastrointestinal symptom, thus showing a significant association between the presence of parasites and diarrhea ( $\chi^2 = 25.12$ ;  $P < 0.0001$ ). The prevalence of *G. lamblia*, *C. parvum*, and *I. belli* was positively and significantly associated with the presence of diarrhea ( $P = 0.037$ ;  $P < 0.001$ , and  $P = 0.036$ , respectively), and an indicative significance ( $P = 0.076$ ) was found regarding *S. stercoralis* infection (Table 3).

## DISCUSSION

The gastrointestinal tract plays a crucial role in AIDS pathogenesis, due to suppressed immunologic responses at the mucosal level that hamper the intestinal non-specific defense mechanisms.<sup>11</sup>

Most of these patients were male young adults, and the HIV-infection was acquired through sexual contact, thus corresponding to the most typical and recent epidemiologic profile of AIDS patients.<sup>3,12</sup>

The diagnosis of intestinal parasites in patients with AIDS was performed by classic parasitologic stool examination, which has been recognized as the key diagnostic tool since the nineteenth century.<sup>13</sup>

It is recognized that three serial stool examinations constitute a more sensitive approach to detect pathogenic intestinal parasites.<sup>14</sup> However, 91% of the single stool examinations are positive, and *C. parvum* oocysts can be found in 96% of the single samples of symptomatic patients.<sup>15,16</sup> In the present study, one single sample was collected from each patient, to study some epidemiologic aspects from this Brazilian population. It is possible that some parasites were not detected in this survey. No other techniques, such as the chromotrope method, for Microsporidia, or adhesive tape or anal swab,

for *Enterobius vermicularis*, were used; therefore, the exact prevalence of intestinal parasites among these patients with AIDS might be underestimated.

The prevalence of patients infected with intestinal parasites was 40%. Most patients were monoparasitized, although 11 (5.5%) presented two or more parasites, and up to four parasite associations could be observed. Usually, *G. lamblia* was found associated with other enteropathogens.

*Giardia lamblia* was the most prevalent parasite (16%) in this 200-sample population, followed by *E. coli* (13%), a protozoa that is not pathogenic for humans, *C. parvum* (7%), *E. nana* (3.5%), a natural inhabitant of the gastrointestinal tract, *A. lumbricoides* (2.5%), *S. stercoralis* (2.5%), *I. belli* (2%), and *Blastocystis hominis* (0.5%).

As diarrhea is an important gastrointestinal symptom in HIV-infected patients, a comparison was conducted between the associated intestinal parasites in diarrheic and nondiarrheic patients, with positive and with negative fecal examinations. Among the fecal samples that did not present parasites, the nondiarrheic group was significantly larger (67.7%) than the diarrheic group (26.7%), showing that the diarrheic states were closely related to the presence of parasites in the stool samples and that diarrhea is an important manifestation of enteroparasitosis. This association is in agreement with other studies showing that only 20% of the diarrheic patients with AIDS presented an obscure etiology, whereas in more than 50% a diagnosis was possible.<sup>17</sup>

The significant association between positive parasitologic examinations and diarrhea was particularly ascribed to the presence of *G. lamblia*, *C. parvum*, and *I. belli* and, in a lesser degree, to *S. stercoralis* infection.

A high prevalence of *C. parvum* was found in the HIV-infected patients with diarrheal stools (24.4%). This rate is higher than those reported in other studies in HIV-patients living in Brazil (between 14% and 17%), and may be ascribed to the selection of diarrheic stools made in the present study, which was not done in the other investigations.<sup>18,19</sup> Cryptosporidiosis is the main cause for chronic diarrhea in HIV-infected patients in industrialized countries, and recent surveys showed that the prevalence of this protozoan ranges between 8% and 23% in the United States and up to 37% in Europe.<sup>20</sup> The official global prevalence of cryptosporidiosis in patients with AIDS reported by the Brazilian Ministry of Health is 0.7%, which is much lower than what was found in this sample (7%).<sup>21</sup> This discrepancy may be explained by the poor conditions in the public laboratories, which do not apply staining techniques, leaving the patient without a precise diagnosis.

Yet, the prevalence of *I. belli* in this investigation agrees with the data reported in other countries. The low prevalence for this protozoan may be linked to the systemic use of trimethoprim-sulfamethoxazole, which is given to

patients with CD4+ cell counts below 200 cells/mm<sup>3</sup> as a prophylactic treatment of pneumocystosis.<sup>6,22-24</sup>

*Giardia lamblia* was the most conspicuous parasite found in both diarrheic (26.7%) and nondiarrheic (12.9%) patients with AIDS. These rates were not surprising, since in some regions of São Paulo state, 40% of the population has *G. lamblia* infection, although frequently these patients bear the cysts in their intestinal tract, without apparent symptoms.

Brazil is a country with a wide diversity of geographic, climatic, economic and social characteristics, and this may reflect the presence of different areas endemic to distinct parasites. All data considered, additional studies should be taken in different areas of Brazil to identify the endemicity of each parasite in the different areas of the country. The present data support the value of standard fecal examinations in HIV-patients, regardless of presenting diarrhea, since these examinations easily can be performed with low costs and frequently elucidate the diagnosis to enable provision of the adequate therapeutic approach.

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